Title: A Proteomic Approach to Identify Peptide Substrates for Sensitive and Specific Detection of Membrane Bound Proteases

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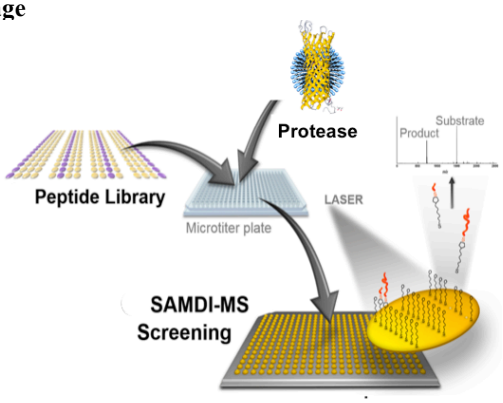
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I. Abstract

Rational design of synthetic peptides has become a critical step during development of functional (cleavage) assays for proteases. In this study we describe a high throughput proteomic methodology to screen for peptides that are effectively and specifically cleaved by the protease. It is based on rationally designed peptide libraries that are screened using Self Assembled Monolayers Desorption Ionization-Mass Spectrometry (SAMDI-MS)[1] to identify potential peptide hits. We start by describing experiments of matrix metalloproteinase-7 (MMP-7)[2] a potential cancer biomarker. However, the focus is on Omptin proteases present on the surface of *E.coli* and *Salmonella*. These membrane proteases, e.g. OmpT and PgtE, serve as a defense mechanism and contribute to the virulence of various pathogenic strains by inactivating/digesting host generated antimicrobial peptides. We demonstrate that the catalytic efficiency (k_{cat}/K_M) of OmpT targeting LL37, an anti-microbial peptide and a natural substrate to OmpT, can be improved ~400-fold by replacing natural cleavage sequence ARRA with and FRRV identified from a SAMDI-MS screening[3]. A portion of the FRRV-modified LL37 peptide was subsequently employed in a fluorescent quenching assay enabling detection of OmpT in detergent micelles down to about 2 nM. It is also shown that it is possible detect at least 10^4 CFU/mL *E.coli* cells with over expressed levels of OmpT in the membrane as well as wild type *E. coli* K12 cells. This is fully comparable to the sensitivity levels obtained by commercial culture-independent test kits opening up for rapid detection of pathogenic strains of *E.coli* and other bacteria.

II. Image



III. References

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IV. Biography

Bo Liedberg received his PhD from Linköping University, Sweden, in 1986, and did an industrial post doc at Pharmacia Biosensor, Uppsala, Sweden, in 1986-1989. He is currently the Director of the Center of Biomimetic Sensor Science and serves also as dean for the Interdisciplinary Graduate School, in Nanyang Technological University, Singapore. His research focuses on self-assembly, flexible devices, bioanalytics and fieldable sensor technologies. He has published more than 300 papers in reputed journals. Liedberg also has been serving as an editorial board member and guest editor for numerous journals.

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